<110> Kocken, Clemens H.M.
      Thomas, Alan W.
      Blackman, Michael J.
      Withers-Martinez, Chrislaine
      Holder, Anthony A.

<120> Efficient expression of Plasmodium apical membrane
      antigen 1 in yeast cells

<130> P54200US00

<140> 10/615,615
<141> 2003-07-08

<150> EP 00204697.7
<151> 2000-12-22

<150> PCT/NL01/00934
<151> 2001-12-21

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<170> PatentIn Ver. 2.1

<210> 1
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<220>
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<220>
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<400> 1
gggggatcca tgagaaaatt atactgcgta tt   32

<210> 2
<211> 40
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: primer PF83B

<220>
<221> misc_feature
<222> (1)..(40)

<400> 2
acgtggatcc ttaatagtat ggtttttcca tcagaactgg   40

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<210> 3
<211> 27
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: primer Pf83A

<220>
<221> misc_feature
<222> (1)..(27)

<400> 3
ggaattccag aactactggg agcatcc                                    27


<210> 4
<211> 29
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: primer Pf83H

<220>
<221> misc_feature
<222> (1)..(29)

<400> 4
gctctagaat gttatcgtaa cgtaggctt                                  29


<210> 5
<211> 32
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: primer Pf83I

<220>
<221> misc_feature
<222> (1)..(32)

<400> 5
gctctagact acatgttatc gtacgtaggc tt                              32


<210> 6
<211> 1869
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Combined DNA/RNA Molecule:
     synthetic gene encoding P. Falciparum AMA-1
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ectodomain with P. pastoris codon usage

<220>
<223> Description of Artificial Sequence: synthetic gene
      encoding P. Falciparum AMA-1 ectodomain with P.
      pastoris codon usage

<220>
<221> CDS
<222> (1)..(1866)

<400> 6
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atg agg aag ttg tac tgc gtt ttg ttg ttg tct gct ttc gag ttc acc   48
Met Arg Lys Leu Tyr Cys Val Leu Leu Leu Ser Ala Phe Glu Phe Thr
 1               5                  10                  15

tac atg atc aac ttc ggt cgt ggt cag aac tac tgg gag cat cct tac   96
Tyr Met Ile Asn Phe Gly Arg Gly Gln Asn Tyr Trp Glu His Pro Tyr
                20                  25                  30

cag aag tct gac gtc tac cat cct atc aac gaa cat agg gag cat cct  144
Gln Lys Ser Asp Val Tyr His Pro Ile Asn Glu His Arg Glu His Pro
            35                  40                  45

aag gaa tac gaa tac cca ctg cat caa gag cac act tac cag cag gaa  192
Lys Glu Tyr Glu Tyr Pro Leu His Gln Glu His Thr Tyr Gln Gln Glu
        50                  55                  60

gat tct ggt gaa gat gaa aac acc ttg caa cac gct tac ccc atc gat  240
Asp Ser Gly Glu Asp Glu Asn Thr Leu Gln His Ala Tyr Pro Ile Asp
 65                  70                  75                  80

cat gaa gga gct gaa cca gcc cct cag gaa caa aac ttg ttc tct tcc  288
His Glu Gly Ala Glu Pro Ala Pro Gln Glu Gln Asn Leu Phe Ser Ser
                85                  90                  95

atc gaa atc gtg gaa aga tcc aac tac atg ggt aac cca tgg act gag  336
Ile Glu Ile Val Glu Arg Ser Asn Tyr Met Gly Asn Pro Trp Thr Glu
                100                 105                 110

tac atg gca aag tac gac atc gag gaa gtg cac gga agt ggt atc agg  384
Tyr Met Ala Lys Tyr Asp Ile Glu Glu Val His Gly Ser Gly Ile Arg
        115                 120                 125

gtt gat ctg ggt gaa gat gcc gaa gtc gct ggt act cag tac aga ctc  432
Val Asp Leu Gly Glu Asp Ala Glu Val Ala Gly Thr Gln Tyr Arg Leu
    130                 135                 140

cct tct ggt aag tgc cct gtt ttc gga aag ggt atc atc atc gaa aac  480
Pro Ser Gly Lys Cys Pro Val Phe Gly Lys Gly Ile Ile Ile Glu Asn
145                 150                 155                 160

tct aag act act ttc ctc aag cct gtt gct act ggt aac caa gat ctt  528
Ser Lys Thr Thr Phe Leu Lys Pro Val Ala Thr Gly Asn Gln Asp Leu
                165                 170                 175

aag gac gga ggt ttc gct ttc cca cct act aac cct ctg atc tct cca  576
```

3

```
Lys Asp Gly Gly Phe Ala Phe Pro Pro Thr Asn Pro Leu Ile Ser Pro
        180             185                 190

atg act ttg aac ggt atg cgt gac ttc tac aag aac aac gaa tac gtc    624
Met Thr Leu Asn Gly Met Arg Asp Phe Tyr Lys Asn Asn Glu Tyr Val
        195             200                 205

aag aac ttg gat gaa ttg act ttg tgt agt aga cac gct gga aac atg    672
Lys Asn Leu Asp Glu Leu Thr Leu Cys Ser Arg His Ala Gly Asn Met
        210             215                 220

aac cct gat aac gac aag aac agt aac tac aag tac ccc gcg gtt tac    720
Asn Pro Asp Asn Asp Lys Asn Ser Asn Tyr Lys Tyr Pro Ala Val Tyr
225             230             235                 240

gac tac aac gat aag aag tgt cac atc ctg tac atc gct gcc caa gaa    768
Asp Tyr Asn Asp Lys Lys Cys His Ile Leu Tyr Ile Ala Ala Gln Glu
            245             250                 255

aac aac gga cca aga tac tgt aac aag gat caa agt aag aga aac tct    816
Asn Asn Gly Pro Arg Tyr Cys Asn Lys Asp Gln Ser Lys Arg Asn Ser
            260             265                 270

atg ttc tgt ttc aga cct gca aag gac aag ctg ttc gaa aac tac gtg    864
Met Phe Cys Phe Arg Pro Ala Lys Asp Lys Leu Phe Glu Asn Tyr Val
        275             280                 285

tac ttg tcc aag aac gtt gtc gat aac tgg gaa gaa gtc tgc cca aga    912
Tyr Leu Ser Lys Asn Val Val Asp Asn Trp Glu Glu Val Cys Pro Arg
        290             295                 300

aag aac ctc gag aac gca aag ttc ggt ctg tgg gtc gat ggt aac tgt    960
Lys Asn Leu Glu Asn Ala Lys Phe Gly Leu Trp Val Asp Gly Asn Cys
305             310             315                 320

gaa gac atc cct cat gtg aac gag ttc agt gct aac gat ttg ttc gag   1008
Glu Asp Ile Pro His Val Asn Glu Phe Ser Ala Asn Asp Leu Phe Glu
            325             330                 335

tgt aac aag ctg gtc ttc gag ttg tct gcc agt gac caa cct aag cag   1056
Cys Asn Lys Leu Val Phe Glu Leu Ser Ala Ser Asp Gln Pro Lys Gln
        340             345                 350

tac gaa cag cat ttg act gac tac gaa aag atc aag gaa gga ttc aag   1104
Tyr Glu Gln His Leu Thr Asp Tyr Glu Lys Ile Lys Glu Gly Phe Lys
        355             360                 365

aac aag aac gcc gat atg atc aag tcc gct ttc ctc cca acc ggt gca   1152
Asn Lys Asn Ala Asp Met Ile Lys Ser Ala Phe Leu Pro Thr Gly Ala
        370             375                 380

ttc aaa gca gat aga tac aag tct cac ggt aag ggt tac aac tgg gga   1200
Phe Lys Ala Asp Arg Tyr Lys Ser His Gly Lys Gly Tyr Asn Trp Gly
385             390             395                 400

aac tac aac aga gaa acc caa aag tgt gaa atc ttc aac gtc aag cct   1248
Asn Tyr Asn Arg Glu Thr Gln Lys Cys Glu Ile Phe Asn Val Lys Pro
```

4

```
acc tgc ctc atc aac gac aag tcc tac att gcg act act gcc ctg tct   1296
Thr Cys Leu Ile Asn Asp Lys Ser Tyr Ile Ala Thr Thr Ala Leu Ser
            420                 425                 430

cat cca atc gaa gtc gaa cac aac ttc ccc tgc agt ctc tac aag gac   1344
His Pro Ile Glu Val Glu His Asn Phe Pro Cys Ser Leu Tyr Lys Asp
            435                 440                 445

gag atc aag aag gaa atc gag cgt gaa agt aag cgt atc aag ttg aac   1392
Glu Ile Lys Lys Glu Ile Glu Arg Glu Ser Lys Arg Ile Lys Leu Asn
            450                 455                 460

gat aac gac gac gaa ggt aac aag aag atc atc gca cct agg atc ttc   1440
Asp Asn Asp Asp Glu Gly Asn Lys Lys Ile Ile Ala Pro Arg Ile Phe
465                 470                 475                 480

atc tcc gat gac aag gat tcc ctc aag tgt cct tgt gac cct gag atg   1488
Ile Ser Asp Asp Lys Asp Ser Leu Lys Cys Pro Cys Asp Pro Glu Met
            485                 490                 495

gtg agt cag tcc act tgt aga ttc ttc gtt tgc aag tgc gtc gaa cgt   1536
Val Ser Gln Ser Thr Cys Arg Phe Phe Val Cys Lys Cys Val Glu Arg
            500                 505                 510

aga gcc gaa gtc act agt aac aac gaa gtt gtc gtg aag gaa gaa tac   1584
Arg Ala Glu Val Thr Ser Asn Asn Glu Val Val Val Lys Glu Glu Tyr
            515                 520                 525

aag gat gaa tac gct gat att cca gag cat aag cct acg tac gat aac   1632
Lys Asp Glu Tyr Ala Asp Ile Pro Glu His Lys Pro Thr Tyr Asp Asn
            530                 535                 540

atg aag atc atc atc gct agt tct gct gct gtc gct gtt ctg gct act   1680
Met Lys Ile Ile Ile Ala Ser Ser Ala Ala Val Ala Val Leu Ala Thr
545                 550                 555                 560

atc ctc atg gtg tac ctt tac aag aga aag gga aac gct gag aag tac   1728
Ile Leu Met Val Tyr Leu Tyr Lys Arg Lys Gly Asn Ala Glu Lys Tyr
            565                 570                 575

gac aag atg gat caa cct caa cat tac ggt aag agt act tcc agg aac   1776
Asp Lys Met Asp Gln Pro Gln His Tyr Gly Lys Ser Thr Ser Arg Asn
            580                 585                 590

gat gag atg ttg gat cca gag gcc tcc ttc tgg ggt gag gag aag aga   1824
Asp Glu Met Leu Asp Pro Glu Ala Ser Phe Trp Gly Glu Glu Lys Arg
            595                 600                 605

gcc tct cat act act cca gtt ttg atg gag aag cct tac tac taa       1869
Ala Ser His Thr Thr Pro Val Leu Met Glu Lys Pro Tyr Tyr
610                 615                 620
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<210> 7
<211> 622

5

<212> PRT
<213> Artificial Sequence
<223> Description of Artificial Sequence: synthetic gene
      encoding P. Falciparum AMA-1 ectodomain with P.
      pastoris codon usage

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Met Arg Lys Leu Tyr Cys Val Leu Leu Leu Ser Ala Phe Glu Phe Thr
 1   5   10   15

Tyr Met Ile Asn Phe Gly Arg Gly Gln Asn Tyr Trp Glu His Pro Tyr
            20   25   30

Gln Lys Ser Asp Val Tyr His Pro Ile Asn Glu His Arg Glu His Pro
        35   40   45

Lys Glu Tyr Glu Tyr Pro Leu His Gln Glu His Thr Tyr Gln Gln Glu
    50   55   60

Asp Ser Gly Glu Asp Glu Asn Thr Leu Gln His Ala Tyr Pro Ile Asp
65   70   75   80

His Glu Gly Ala Glu Pro Ala Pro Gln Glu Gln Asn Leu Phe Ser Ser
                85   90   95

Ile Glu Ile Val Glu Arg Ser Asn Tyr Met Gly Asn Pro Trp Thr Glu
            100   105   110

Tyr Met Ala Lys Tyr Asp Ile Glu Glu Val His Gly Ser Gly Ile Arg
        115   120   125

Val Asp Leu Gly Glu Asp Ala Glu Val Ala Gly Thr Gln Tyr Arg Leu
    130   135   140

Pro Ser Gly Lys Cys Pro Val Phe Gly Lys Gly Ile Ile Ile Glu Asn
145   150   155   160

Ser Lys Thr Thr Phe Leu Lys Pro Val Ala Thr Gly Asn Gln Asp Leu
                165   170   175

Lys Asp Gly Gly Phe Ala Phe Pro Pro Thr Asn Pro Leu Ile Ser Pro
            180   185   190

Met Thr Leu Asn Gly Met Arg Asp Phe Tyr Lys Asn Asn Glu Tyr Val
        195   200   205

Lys Asn Leu Asp Glu Leu Thr Leu Cys Ser Arg His Ala Gly Asn Met
    210   215   220

Asn Pro Asp Asn Asp Lys Asn Ser Asn Tyr Lys Tyr Pro Ala Val Tyr
225   230   235   240

Asp Tyr Asn Asp Lys Lys Cys His Ile Leu Tyr Ile Ala Ala Gln Glu
            245   250   255

Asn Asn Gly Pro Arg Tyr Cys Asn Lys Asp Gln Ser Lys Arg Asn Ser
            260   265   270

6

```
Met Phe Cys Phe Arg Pro Ala Lys Asp Lys Leu Phe Glu Asn Tyr Val
        275             280             285

Tyr Leu Ser Lys Asn Val Val Asp Asn Trp Glu Glu Val Cys Pro Arg
        290             295             300

Lys Asn Leu Glu Asn Ala Lys Phe Gly Leu Trp Val Asp Gly Asn Cys
305             310             315             320

Glu Asp Ile Pro His Val Asn Glu Phe Ser Ala Asn Asp Leu Phe Glu
            325             330             335

Cys Asn Lys Leu Val Phe Glu Leu Ser Ala Ser Asp Gln Pro Lys Gln
        340             345             350

Tyr Glu Gln His Leu Thr Asp Tyr Glu Lys Ile Lys Glu Gly Phe Lys
    355             360             365

Asn Lys Asn Ala Asp Met Ile Lys Ser Ala Phe Leu Pro Thr Gly Ala
    370             375             380

Phe Lys Ala Asp Arg Tyr Lys Ser His Gly Lys Gly Tyr Asn Trp Gly
385             390             395             400

Asn Tyr Asn Arg Glu Thr Gln Lys Cys Glu Ile Phe Asn Val Lys Pro
            405             410             415

Thr Cys Leu Ile Asn Asp Lys Ser Tyr Ile Ala Thr Thr Ala Leu Ser
        420             425             430

His Pro Ile Glu Val Glu His Asn Phe Pro Cys Ser Leu Tyr Lys Asp
        435             440             445

Glu Ile Lys Lys Glu Ile Glu Arg Glu Ser Lys Arg Ile Lys Leu Asn
    450             455             460

Asp Asn Asp Asp Glu Gly Asn Lys Lys Ile Ile Ala Pro Arg Ile Phe
465             470             475             480

Ile Ser Asp Asp Lys Asp Ser Leu Lys Cys Pro Cys Asp Pro Glu Met
            485             490             495

Val Ser Gln Ser Thr Cys Arg Phe Phe Val Cys Lys Cys Val Glu Arg
        500             505             510

Arg Ala Glu Val Thr Ser Asn Asn Glu Val Val Val Lys Glu Glu Tyr
        515             520             525

Lys Asp Glu Tyr Ala Asp Ile Pro Glu His Lys Pro Thr Tyr Asp Asn
    530             535             540

Met Lys Ile Ile Ile Ala Ser Ser Ala Ala Val Ala Val Leu Ala Thr
545             550             555             560

Ile Leu Met Val Tyr Leu Tyr Lys Arg Lys Gly Asn Ala Glu Lys Tyr
            565             570             575
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```
Asp Lys Met Asp Gln Pro Gln His Tyr Gly Lys Ser Thr Ser Arg Asn
        580             585             590

Asp Glu Met Leu Asp Pro Glu Ala Ser Phe Trp Gly Glu Glu Lys Arg
        595             600             605

Ala Ser His Thr Thr Pro Val Leu Met Glu Lys Pro Tyr Tyr
    610             615             620
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